ITERG

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INNOVANT PAR NATURE

- **CONTEXT**

Using alternative methods in animal testing is a **necessity** (ethical approach).

EFSA: decrease and improve testing on laboratory animals

→ promotes alternative approach when possible. Directive 2010/63/EU: "3Rs" Aim: "Replacement, Reduction and Refinement".

Study the intestinal absorption of a lipid/proteic compound and its fate in the organism

→ **various chemical, enzymatic and mechanical phenomena, under the effect of complex regulatory pathways in vivo**

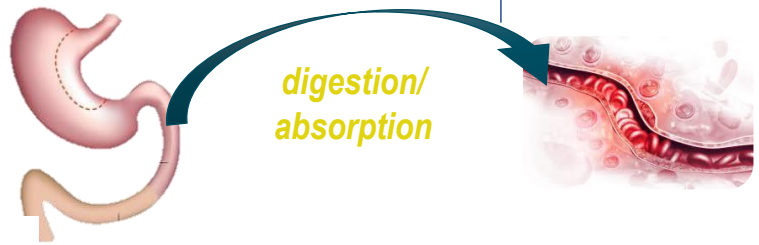
→ different approaches for studying the digestion of a compound: in vivo, in vitro or in silico models/methods

- Experimental studies in animals or clinical trials in humans remain the best approach.

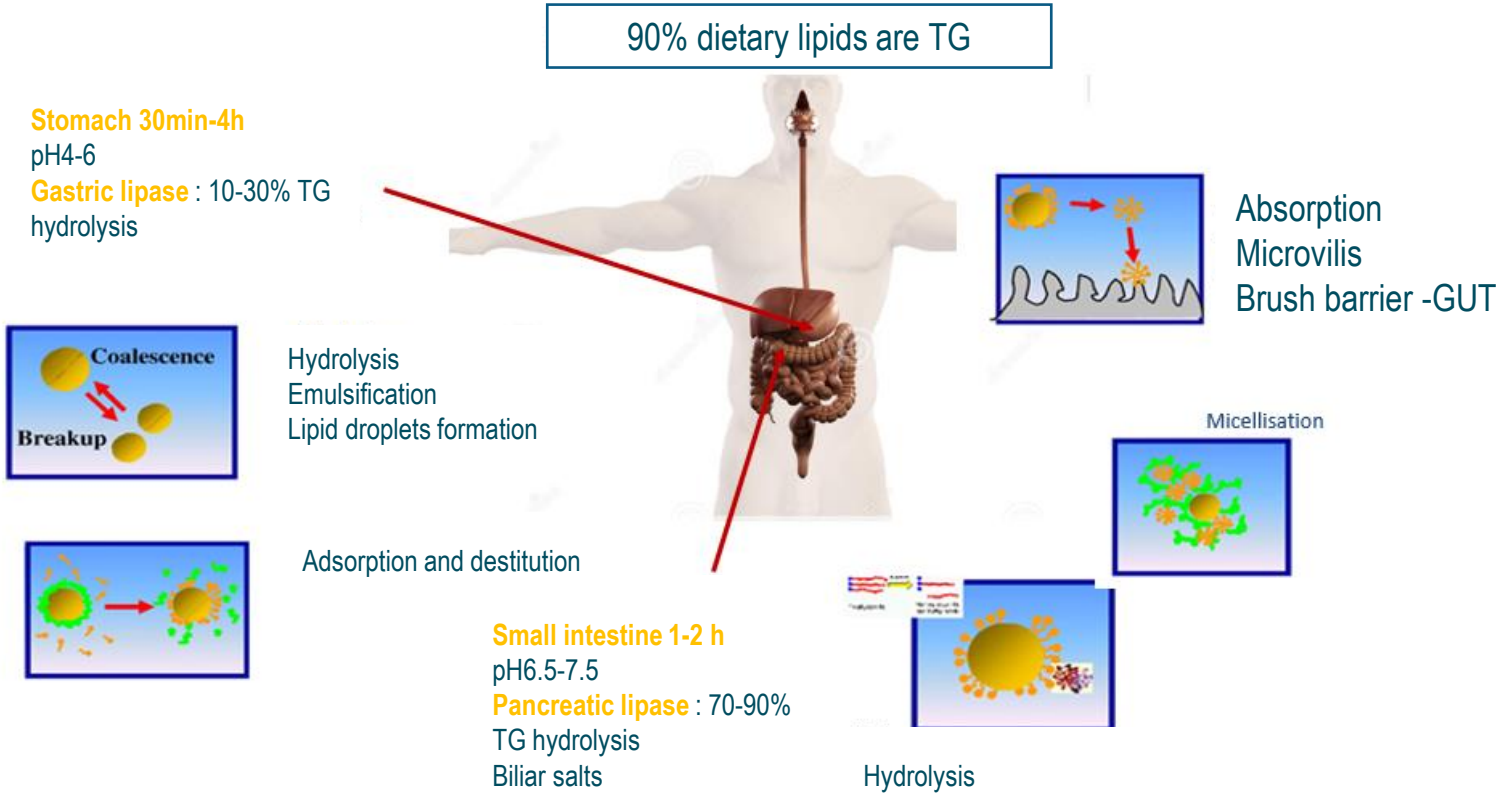
→ Clinical digestion studies in humans are ethically and technically difficult to set up.
→ Studies are cost effective and difficult to carry out in large numbers.
→ *Alternative by in vitro-ex vivo methods should be used*

Problem: take into account the differences between these models → they do not reproduce the biological complexity of the digestive tract and its metabolism.

☝ **It is important to be aware of the advantages and limitations of the different approaches to comply the objectives of the study related to the uptake and metabolic fate of specific molecules.**



- Objective: to use in vitro and/or in vivo models under physiological conditions as close as possible to those in vivo – **lipid digestion**



Based on in vivo data :

- pH variations
- Several Enzymes / concentrations

- **The lipolysis kinetics of lipid products** was followed according to the model established by the consortium **INFOGEST** (Minekus Brodgorb model).

- **Round-robin Studies** from several European laboratories → A unique protocol to study the lipid-protein digestion

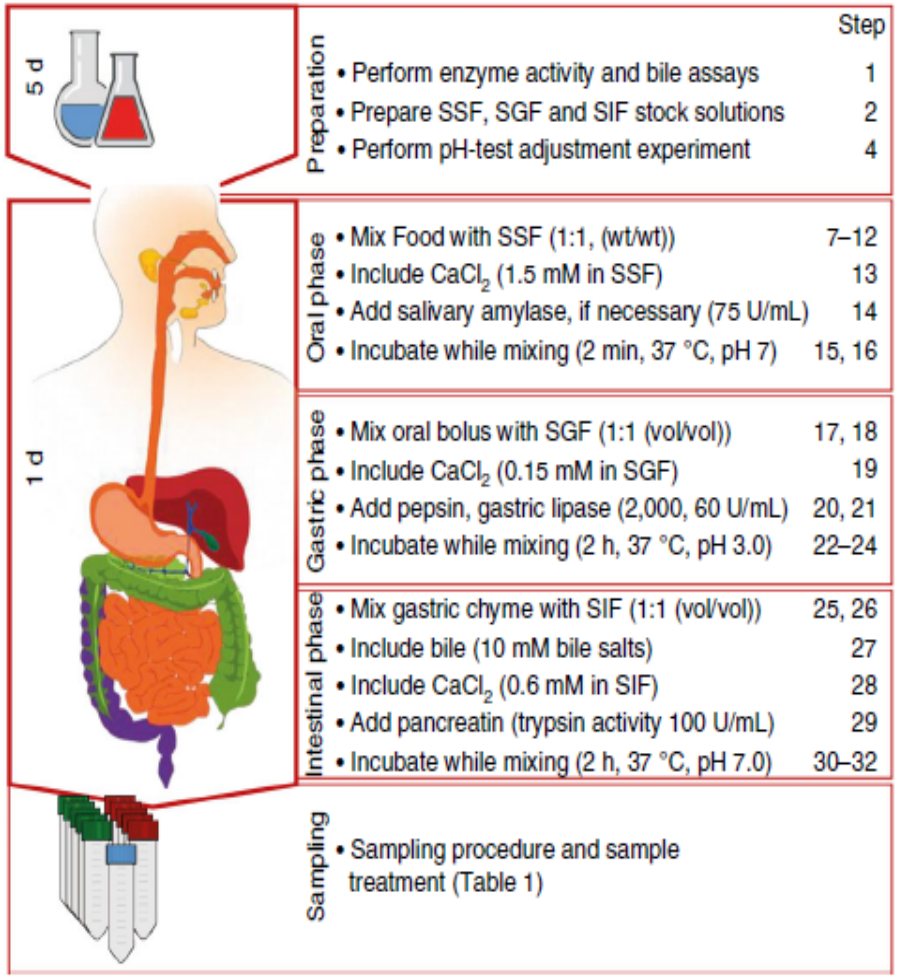
- **This protocol implements 3 types of consecutive digestion :**
- Oral / gastric and Intestinal steps

Proteins and Lipids are subject to GI digestion → the entire enzymatic apparatus for lipids (gastric lipase and pancreatin) + cofactors at pH : *pHstat modele titration).*

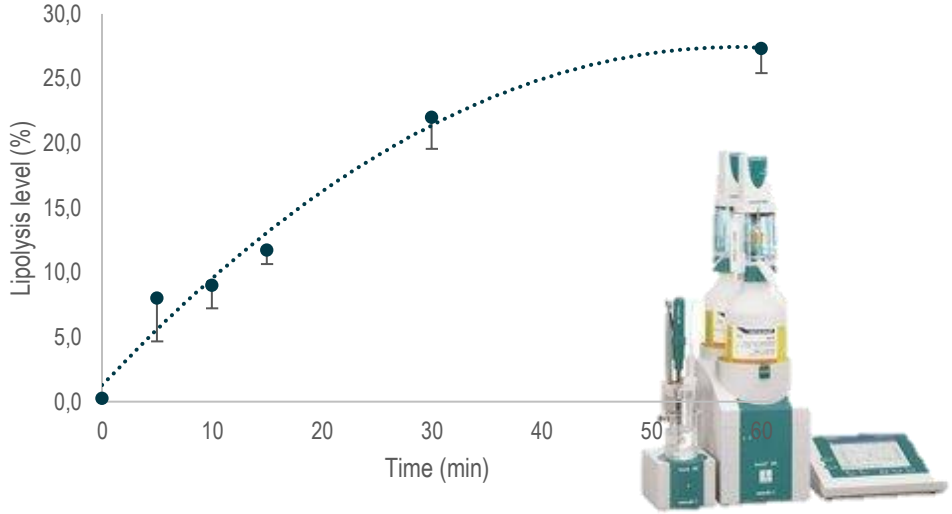
- **Lipolysis level =** quantification of hydrolysates and intermediates of (FFA, MG, DG) from TG lipolysis.

- **Proteolysis level** = quantification of hydrolysates and intermediates of (Am Ac and peptides) from protein hydrolysis

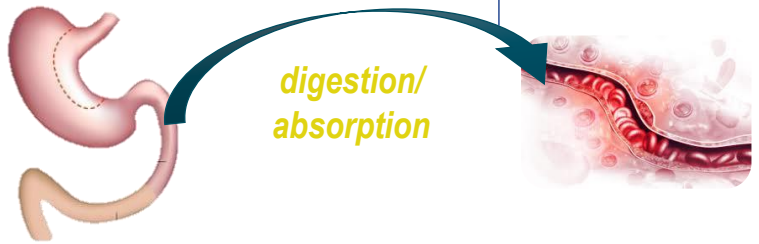
I. Study of an in vitro lipid digestion model



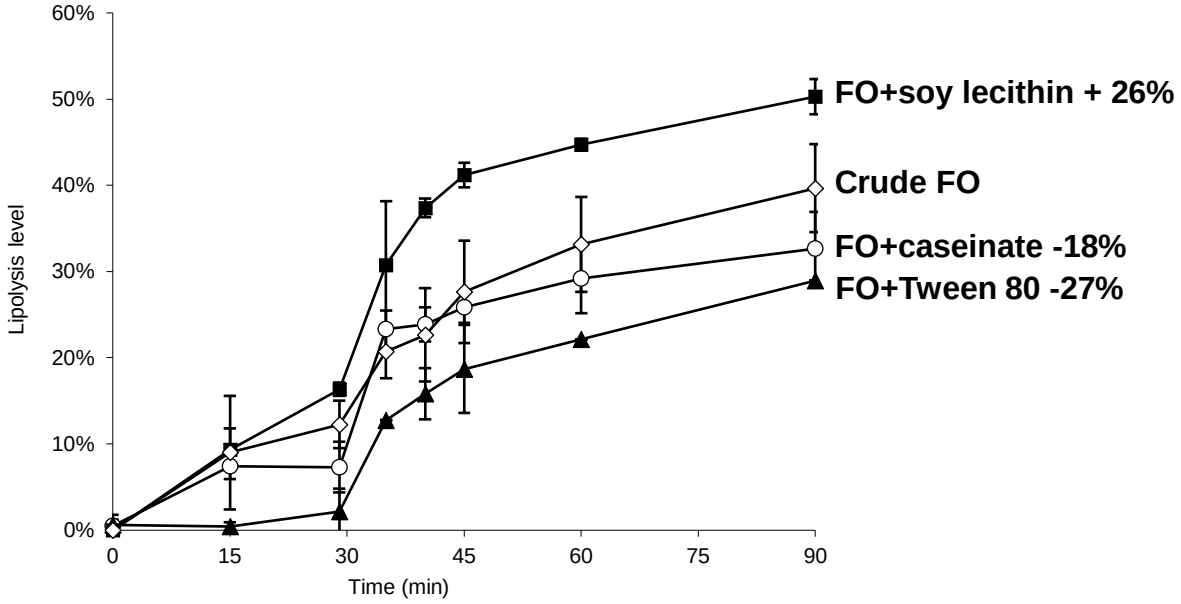
Flow chart of the INFOGEST 2.0 in vitro digestion method for foods. SGF, simulated gastric fluid; SIF, simulated intestinal fluid; SSF, simulated salivary fluid (after Brodgorb et al .2018).



- In the consortium: monitore the optimisation steps and validation of proportions, ratios etc. → validation of the in vitro lipid digestion model: publication of the work



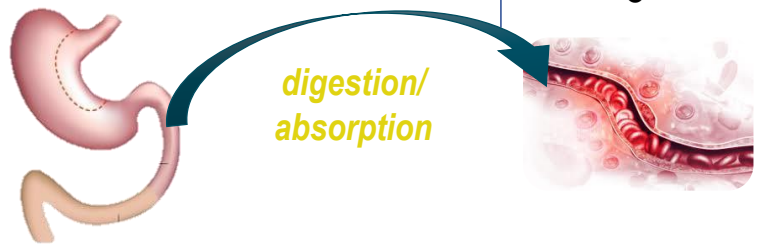
- *Application example: study of the GI digestibility of a lipid emulsion with surfactants of different nature*



Lipolysis levels of flaxseed oil in crude phase (◊) or emulsified with sodium caseinate (○), soy lecithin (■) or Tween 80 (▲). According to Couedelo et al,, 2015-LISACarnot study.

➢ **In vitro study: screen and an emulsifier for nutritional application:**

- **better absorption** of specific compounds by emulsion lipid carriers.
- **Lipid digestibility** of a formula
- **Produce hydrolysates for** cell culture to study their intestinal absorption –micelle formation

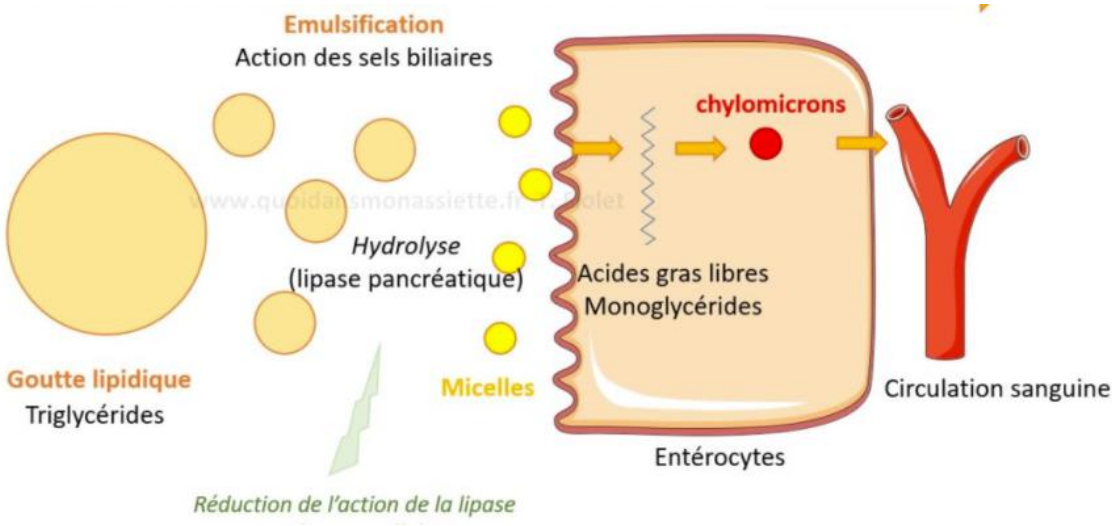


➢ ***Development of an in vitro model of intestinal absorption of lipids and fat-soluble nutrients***

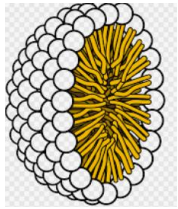
- **Hydrolysis and micellisation of nutrients**

fat-soluble compounds must be hydrolysed → in vitro lipolysis step for fat-soluble compounds as my in vivo conditions

→ pre-digest the compounds to make them "assimilable" by the intestinal cells in the form of mixed lipid-bile salt micelles



- **Optimisation of mixed lipid/bile salt micelles**



✓ In vitro lipolysis of systems - In vitro action of lipolytic enzymes (pancreatin: pancreatic lipase + CEH)

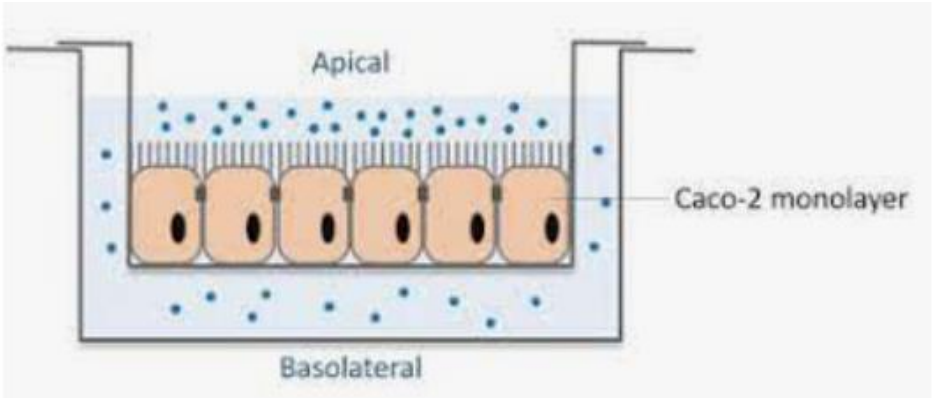
✓ Extraction and micellisation of hydrolysis products (GLA, MG and DG) in nutrient medium + bile salts.

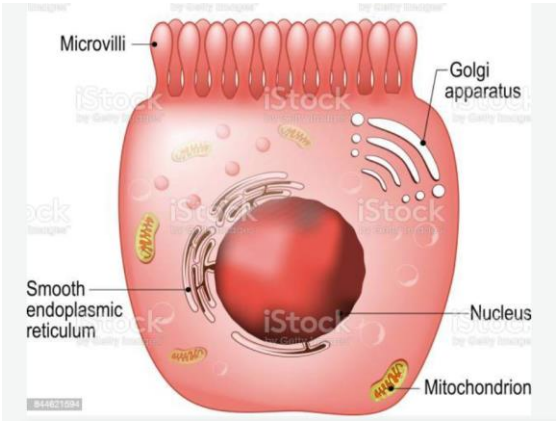
➢ Study of the target value of bile salts and lipids in same bile salts → effect on cytotoxicity

➢ **Study of intestinal absorption of compounds in vitro :**
use of a human adenocarcinoma cell line (Caco-2) in the colon (similar structure with in vivo gut)

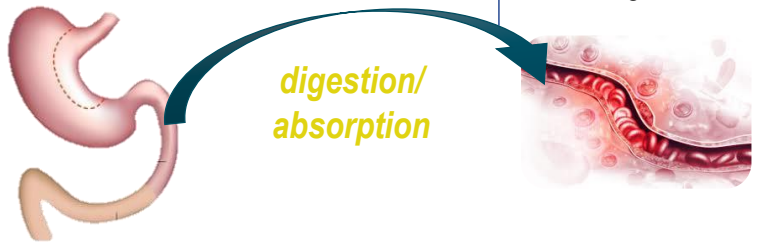
- Cellular differentiation → structural and functional characteristics similar to healthy mature enterocytes.
- At the confluence → monolayer of polarised cells
- Apical pole, tight junctions and a brush border → structure with microvilli comparable to healthy mature enterocytes.

These cell lines make it possible to study :
- **the absorption of compounds through the intestinal wall**
- **Their impact on cellular toxicity**
- **enterocyte functions, including those of lipid-protein metabolism**



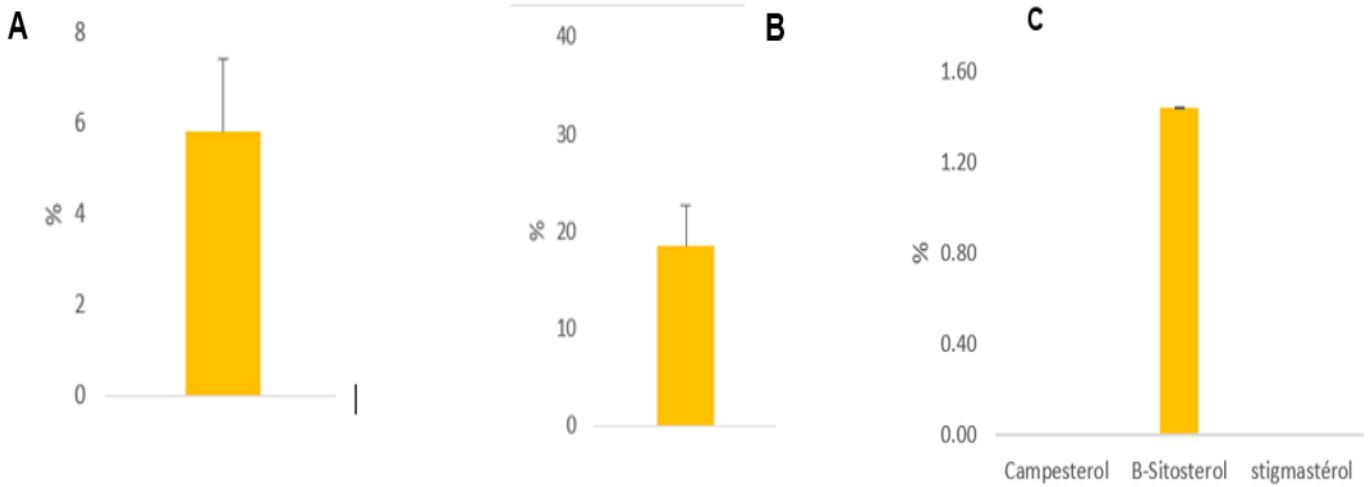


- **Two compartments (apical and basal-lateral compartment)**
- **→ allows the rapid screening of different molecules at the same time.**



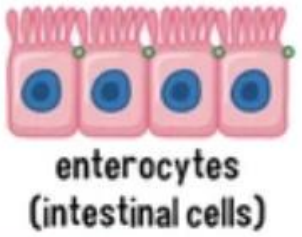
- lipid micelles were made from vegetable oil, on the fatty acids that the phytosterols and fat-soluble vitamins

  - **Vegetable oil**

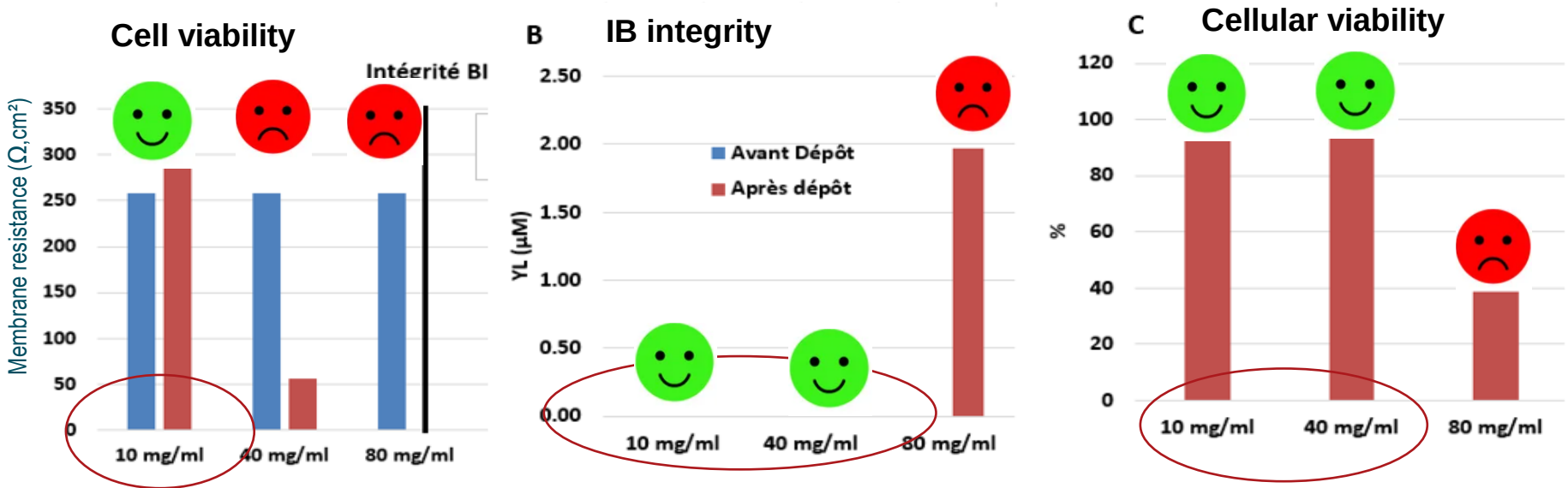


Uptake of FAs (A), α-tocopherol (B) and phytosterols (C) 4 hours after oil deposit on Caco-2 HT27 cells (P7).

- fatty acid absorption rate of sunflower oil : 6%
- absorption rate of α-tocopherol : 18.2%
- low passage rate of phytosterols (campesterol, β-sitosterol and stigmasterol) → only β-sitosterol was absorbed (uptake is < 2%)



➢ Protein hydrolysates



- Impaired BI integrity for > 40 mg/ml (80%)
- Alteration of paracellular permeability and cell viability at 80 mg/ml digestate concentration

Cell viability (A), BI integrity (B) and paracellular permeability (C) as a function of protein digest amounts deposited at the apical pole of Caco-2 cells

➢ Validation of a protocol for protein digestate concentrations

CONCLUSION

The objective is to define different alternative methods to animal studies that respond to in vivo study,
→ digestion and absorption of lipidic and proteic compounds and that allow to free all or part of an approach on animal model.

Beforehand → to take into account the various stages upstream that constitute the digestion. → **Key and unavoidable step (chemical, enzymatic and mechanical phenomena at the same time).**

Implementation of different methods in the laboratory:

- ***Model of gastrointestinal digestion of nutrients in vitro***

✓ Lipolysis of systems - study of the digestibility of lipids and liposoluble molecules according to their formulation and follow the hydrolysates quantitatively and qualitatively of the hydrolysed nutrients (MG, GLA and residual DG)

✓ Proteolysis and digestibility of proteins (peptides, amino acids),

✓ GI digestion of nutrients: a prerequisite for cell culture methods for micellisation by reproducing in vitro the physiological conditions of digestion in the gastrointestinal tract.

→ **in the framework of the INFOGEST consortium → optimization of gastrointestinal conditions that best mimic human physiology.**

- **Preparation of systems in contact with cells or tissues in culture (lipids and proteins)**

- ***Cell model - caco-2 cells (cells isolated from a human colonic adenocarcinoma)***

To screen and compare several formulas on :

- Their cellular toxicity and impact on the quality of the intestinal barrier.
- Intestinal absorption of compounds of interest
- Cellular mechanisms involved

→ Model could also be used as a complement to in vivo experimentation (animal or human) to minimize in vivo studies

THANK YOU FOR YOUR ATTENTION